MODIFIED AGAROSE AND AGAR AND METHOD OF MAKING SAME

application is a continuation of Ser. No. 150,778 filed June 7, 1971, now abandoned.

This invention relates to modified agarose and agar having lower gelling and melting temperatures than those of the corresponding unmodified agarose and agar, and to a method of modifying agarose and agar to lower their gelling and melting temperatures.

Both agar and agarose (one of the constituents of agar) in the form of aqueous gels have been widely used as culture media and as substrates for electrophoresis and for diffusive interactions of various kinds; agarose gels offer particular advantages because of their essentially non-ionic nature. In all such applications, as well as in the use of agar and agarose as thickeners in foods, cosmetics, and in other conventional uses of these materials, the gelling temperature is of particular importance. For example, although the gelling temperature of solutions of many samples of agar and agarose obtained from natural sources is about 35°–36°C., long standing of such solutions at a temperature of 40°C. or even higher frequently results in thickening or incipient gelation. In using such solutions for biological assays, it is therefore difficult to prepare specimens without operating at temperatures so high as to kill or inactivate the organisms or reagents employed.

Study of the varying methoxyl content of agarose obtained from natural sources has shown that samples having higher methoxyl content also have higher gelling temperatures.

It has now been discovered that by modifying the agarose by increasing the methoxyl content through a methylation reaction, the gelling temperature of agarose is lowered instead of raised. It has also been found that similar lowered gelling temperatures are obtained in the case of alkylated, alkenylated, acylated and hydroxyalkylated agarose in which the alkyl, alkenyl and acyl groups contain up to three carbon atoms and the hydroxyalkyl groups contain up to four carbon atoms. Various alkylation, alkenylation, acylation or hydroxyalkylation reactions may be employed to achieve the desired results.

The term "gelling temperature" as used herein means the temperature at which a liquid or sol hardens into a rigid gel upon cooling and is to be distinguished from gelatinization which involves hydration, as in the case of starch, for example. In particular, "gelling temperature" means the temperature at which hardening occurs when a solution containing 1.5% by weight of agarose or agar is cooled at the rate of 0.5°C. per minute.

The present invention is also useful with agar, which contains, along with agarose, agaropectin as well, since it is the agarose fraction which is responsible for gelation. Because no ionic groups are introduced into agar or agarose by the present invention, it is of particular value for providing agarose of low gelling temperature for use as a substrate in electrophoresis. A decrease in gelling temperature to as low as the freezing point of water or even lower can be achieved by the present invention.

Other important properties of agar and agarose are also improved by the present invention: melting temperature is decreased and clarity of the gel is increased.

The extent of alkylation, alkenylation, acylation or hydroxyalkylation required to achieve a specific extent of lowering of the gelling temperature varies depending to some extent upon the source and original gelling temperature of the unmodified agar or agarose as well as upon the identity of the particular alkyl, alkenyl, acyl or hydroxyalkyl group present. The extent of substitution can be defined in terms of the four theoretically available sites for reaction which are present in the disaccharide molecule composed of D-galactose and 3,6-anhydro-L-galactose, which disaccharide is the principal component of agarose. On this basis, a product in which all of the available sites have been completely reacted is a product having a degree of substitution (D.S.) of 4.0. As a specific example, the increase in D.S. of a hydroxyethylated product in accordance with the present invention can be computed as follows:

$$\text{Increase in D.S.} = \frac{306 \times \text{wt. percent hydroxyethyl}}{(100 \times 45) - (44 \times \text{wt. percent hydroxyethyl})}$$

In general, products of the present invention have an increase in D.S. from about 0.01 to about 1.0; while it is possible to achieve a greater increase in D.S., there is little advantage to an increase beyond 1.0. The minimum useful increase in degree of substitution for the most desirable and most widely useful products is that required to provide a modified agar or agarose having a gelling temperature at least 1°C. lower than that of the corresponding unmodified agar or agarose; as can be seen from the data given in the following examples, this amounts to an increase in D.S. of at least about 0.01, depending upon the particular substituent group present.

In carrying out the process of the present invention in an aqueous medium, as is generally preferred, the agar or agarose is first dissolved in strong aqueous alkali, about 0.5 to 1.5 molar in alkali metal hydroxide, after which a suitable reagent is added, such as dimethyl sulfate, ethyl bromide, 1-bromopropane, 2-bromopropane, 3-bromopropene, propylene oxide, ethylene oxide, 2-chloroethanol, epichlorohydrin, butylene oxide, diepoxybutane, and the like. Since some discoloration or darkening of the solution tends to occur during the reaction when it is carried out in aqueous alkaline solution, producing a product which is discolored although otherwise entirely satisfactory, it is also preferred to block the aldehyde end group of the agarose, for example by reduction, before bringing the agar or agarose into contact with aqueous alkali, thus preventing the color-forming reaction which involves the aldehyde group from taking place. The blocking agent of choice is a borohydride, particularly an alkali metal borohydride such as sodium borohydride, which reduces the aldehyde end group to an alcohol (hydroxy) group.

A difunctional reagent such as epichlorohydrin which is capable of producing cross-linking under appropriate conditions to form a water-insoluble product can be used only under conditions which prevent cross-linking and which result in a water-soluble product, i.e., soluble to the extent of at least 2% by weight at 90°C. As is well known, cross-linking can be avoided by employing a dilute (less than about 3.5% by weight) solution of agar or agarose for the reaction and by other techniques well known to those skilled in the art. Except for the necessity of avoiding formation of a water-